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## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Terry et al.

Serial No. 09/365,349

Filed: July 30, 1999

For: *Heavy Metal Phytoremediation*

Group Art Unit: 1638

Examiner: Ibrahim, M.

Attorney Docket No. B99-085

## CERTIFICATE OF TRANSMISSION

I hereby certify that this corr. is being transmitted by fax to the Comm. for Patents, Washington, D.C. 20231 at 703 305-7401 on September 21, 2000.

Signed

  
Richard OsmanRESPONSE

The Commissioner for Patents  
Washington D.C. 20231

Dear Examiner Ibrahim,

Thank you for the telephone call of 09/19/00, wherein it was queried whether the claimed methods are enabled as applied to plants or just to one particular type of plant, Brassicaceae.

The claims are directed to a plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provides enhanced heavy metal accumulation as compared with a corresponding wild type plant; and to a method for decreasing heavy metal content of a medium (such as soil), comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a subject plant in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased. See specification, p.3, lines 23-26 and pending claims.

The specification teaches that "a wide variety of plants may be used, as urged by the particular trace element, medium, site geology, topology, weather, etc. Additional factors for selection include large biomass production, relatively high trace element accumulation capacity, and ease of genetic engineerability (Zhu et al., 1999, Plant Physiol 119:73-79). Suitable plants are readily screened for requisite engineerability and expression from exemplars of candidate plant varieties by those skilled in the art of plant genetic engineering, as exemplified below."

Specification, p.4, lines 6-11. The specification offers a large number of suitable commercially available varieties of exemplary plant source materials (p.4, line 11 - p.6, line 9). Furthermore, the specification describes diverse exemplary plant species demonstrating enhanced heavy metal accumulation in wild-type plants (wt) and the corresponding plant overexpressing recombinant glutamylcysteine synthase (r) (p.7, line 26 - p.8, line 18), including *Brassica juncea*, *Populus angustifolia*, *Nicotiana tabacum* and *Silene cucubalis*. The suitability of any given plant is readily ascertained by simple substitution into the same method.

The invention is premised on Applicants' finding that the recited glutamylcysteine synthase effects heavy metal accumulation, is causative of heavy metal accumulation and is rate-limiting of heavy metal accumulation. The disclosure establishes a predictable relationship between heavy metal exposure and overexpression of glutamylcysteine synthase; namely, that such overexpression promotes enhanced accumulation of the metal. This relationship is shown to hold across numerous and diverse exemplary plant species (*supra*). Accordingly, the specification aptly enables one of ordinary skill in the art to practice the method in any plant which is genetically engineered to overexpress glutamylcysteine synthase and thereby provide enhanced accumulation of the heavy metal.

The uncertain and unpredictable relationship cited in our Appeal Brief (e.g. p.4, line 24) relates not to the subject methods or their extrapolation to various plants, but rather to the prior art establishment of an uncertain and unpredictable relationship between glutamylcysteine synthase expression and heavy metal exposure. As explained in the Brief, Chen et al. (1994) report that mutant tomato cells selected for cadmium tolerance show increased ECS activity.

A detailed reading of Chen and subsequent work from Chen's laboratory (not cited in the Action) reveals that the prior art not only fails to suggest the claimed invention, but in fact teaches directly away from it. In their discussion section, Chen acknowledges that the relationships between ECS activity, glutathione synthetase (GS) activity, phytochelatin (PC) synthesis, heavy metal tolerance and heavy metal accumulation are by no means clear. While Chen's results are similar to those of Steffens et al. (1989), cited by Chen on p.238 col 1, lines 50-53, other published reports suggest the opposite. For example, at p.238, col 2, line 20-25 Chen also cites de Knecht et al. (1992) for demonstrating that Cd-tolerant plants can synthesize fewer PCs than sensitive plants exposed to the same Cd concentration. Other data cited by Chen

suggest that this mechanism of Cd-tolerance may not provide a practical route for generating useful plants. First, Chen's Cd-tolerance is not stable (Chen, p.238, col 1, lines 12-14) and second, such metal tolerant plants demonstrate poor growth characteristics (Chen, p.238, col 1, lines 22-25). Chen concludes by suggesting that future development of transgenic plants with altered capacities to synthesize either GSH or PCs might be used to test their hypothesis that increased GSH and/or PC synthesis increases Cd tolerance.

The senior author of Chen et al. subsequently reported on exactly these experiments (see our Specification, p.3, lines 5-9 and the Goldsbrough, 1999, reference cited therein) and like Arisi's poplars, Goldsbrough's transformed *Arabidopsis* plants provided no increase in heavy metal accumulation compared with controls. Specifically, Goldsbrough reports that while ECS could restore some degree of Cd tolerance to a Cd-sensitive mutant (a *cad2* mutant having reduced GSH levels), this gene did not increase Cd tolerance of wild type plants (Goldsbrough, p.230, line 35)<sup>1</sup>. Interestingly, Goldsbrough also further confounds the teachings of Chen by reporting that the ECS gene does not show any change in RNA expression in plants or cells that are exposed to Cd (Goldsbrough, p.230, lines 28-30).

The prior art does not suggest modifying Raskin to overexpress ECS, rather than an MT, and thereby secure a plant providing enhanced heavy metal accumulation. The prior art establishes an uncertain and unpredictable relationship between ECS expression and heavy metal accumulation, and specifically teaches (in both Noctor et al. and Goldsbrough) that over expression of ECS will not yield heavy metal accumulators.

What these references show is that simple upregulation of a gene (such as ECS) in response to cultivation in the presence of a heavy metal does not suggest that the plant will demonstrate enhanced accumulation of the heavy metal. The prior art establishes an uncertain and


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<sup>1</sup> The final action suggests that the positive result with the Cd-sensitive mutants supports the rejection. We believe that would only be true if the claims encompassed plants which accumulate normal amounts of heavy metal. However, the present invention and pending claims do not relate to sensitive mutant plants restored by genetic engineering to accumulate normal amounts of heavy metal. The invention relates to hyper-accumulators. The claims now clarify that enhanced means enhanced over normal, wild-type accumulation - the claims do not and were never intended to encompass a Cd-sensitive mutant engineered to provide merely normal, wild-type heavy metal accumulation.

unpredictable relationship between glutamylcysteine synthase and heavy metal exposure, and specifically teaches (in both Noctor et al., Goldsbrough and Terry) that overexpression of enzymes upregulated upon heavy metal exposure, including glutamylcysteine synthase, will not yield heavy metal accumulators.

This unpredictability relates to extrapolating from gene upregulation to metal accumulation and has no bearing on substituting one plant for another in the claimed methods, wherein enhanced accumulation is demonstrated – in fact, it is demonstrated in a variety of diverse exemplary plants. The specification aptly enables one of ordinary skill in the art to practice the method in any plant which is genetically engineered to overexpress glutamylcysteine synthase and thereby provide enhanced accumulation of the heavy metal.

Respectfully submitted,  
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